INSTITUT NEUROMED
Zentrum für klinische Studien in der Neurologie

Parkinson's Disease Biomarker Program

Evaluation of novel EVaSyn-based biomarkers in a clinical study to assess their sensitivity and specificity as diagnostic and prognostic markers

Cooperation partners:
- Primarius Dr. Andreas Winkler
- Prof. Dr. med. Dirk Strunk

Dr. Strunk has established a novel method to measure nano-sized Extracellular Vesicles (EV) that enables to determine aSyn pathology in human beings (key references below). This has the potential of early diagnosis and of mirroring the course of the disease. Once these features are established, the potential of the markers to serve as indicators of response to treatment with novel disease-modifying agents would be the next level of development.

Dr. Winkler, a renowned neurologist, experienced in the set-up and execution of clinical studies in various neurological indications, and his team would provide the clinical infrastructure required for the evaluation of the EVaSyn-based biomarker platform.

The envisaged prospective clinical study will collect biological samples and clinical information on patients with Parkinson's disease and other neurological disorders such as Alzheimer's disease and multiple sclerosis. Healthy age-matched individuals will serve as another specificity control group. The first analysis informing on sensitivity and specificity of the biomarkers will be applied to the cohort in a cross-sectional manner. To learn on the prognostic significance of the EVaSyn-based biomarkers, they will be tested in longitudinal on the same cohort as well.

EVaSyn-inspired Biomarker Program

Why Biomarkers?

A diagnostic biomarker is a measure for any aspect in the body associated with the presence of disease. Most clinical trials use symptom assessment, such as the Unified Parkinson's Disease Rating Scale (UPDRS), to determine treatment efficiency. Advanced brain imaging (e.g., DaTscan) can help measure Parkinson's disease (PD) in earliest stages, but no widely available and affordable biomarker tests have been conclusively validated (The Michael J. Fox Foundation for Parkinson's Research; Fig.1). Ideally, a diagnostic biomarker can detect PD in advance of symptom onset.

Figure 1: Biomarker Discovery *is aiming to utilize any measurement (blood or cerebrospinal fluid test, imaging, biopsy + histopathology, etc.) to identify candidate biomarkers which need to be verified in patients vs. healthy individuals before initiating assay development. Assay validation occurs in clinical trials followed by extensive long-term qualification in thousands of individuals. (Modified from* ***Johns Hopkins Univ. PD Biomarker Program****)*

Most PD patients are diagnosed due to overt symptoms after disease onset or progression. Reliable PD biomarkers are urgently needed for improved diagnosis and to facilitate monitoring of disease-modifying therapies. The laboratory of Prof. Strunk initiated a PD biomarker discovery program. Based on extended experience in EV research, they recently started to verify and validate extracellular vesicle-associated aSyn as a potential biomarker, in blood or cerebrospinal fluid, to predict PD onset and thus allow for selecting patients at PD risk for vaccination. Extending this strategy, they propose to challenge (1) patient-friendly non-invasive bio-fluids (tears, saliva, urine), (2) using novel nano-vesicle-proteomics, (3) in fractionated bio-fluid at highest sensitivity, to discover **NEW PD BIOMARKERS.** Their most recent unpublished data already indicate that biomarker-testing on nano-sized EVs using their novel EV analysis technology is highly sensitive and shows preferential enrichment of pathologic aSyn (Fig.2).

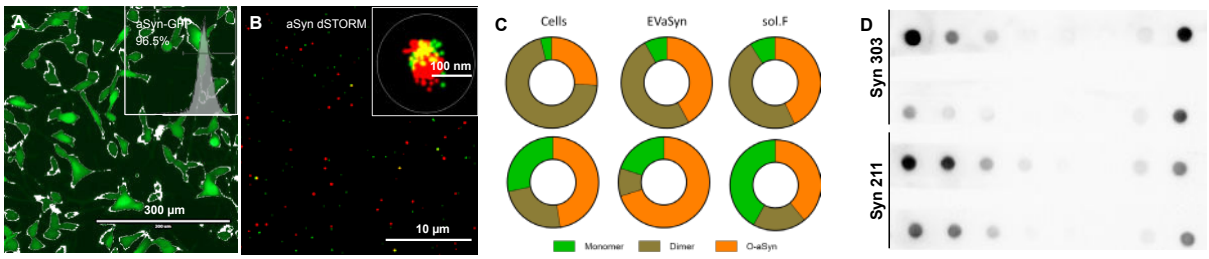

Figure 2: EvaSyn theranostic biomarker development. *(A) Test cell line showing >96% aSyn-GFP. (B) Super-resolution microscopy of EVaSyn pilot reparation showing tetramix CD9/63/81 tetraspanin+ EVs in red, EVaSyn in yellow and free aSyn in green; insert showing one magnified EV. (C) Pilot data indicating preferential enrichment of aggregated (oligomeric) OaSyn on EV. D) ECL titration of two reference antibodies (Syn303, Syn211) towards assay development (**D. Strunk, unpublished data**).*

Dr. Winkler – ongoing clinical studies & publications:

- *MPULSE 2: StIMulation of brain Plasticity to improve Upper Limb recovery from StrokE. A prospective pilot study (case series) to assess efficacy and safety of neuroplastic intervention by Cerebrolysin and atDCS on motor function recovery in subacute and chronic stroke patients*
- *ADVANCE – A Phase 2b, Multicenter, Randomized, Placebo-controlled, Double-blind Study to Assess the Safety and Efficacy of AD04 in Patients with Early Alzheimer's Disease – ADVANCE*
- *Use of Nexstim Navigated Brain Therapy (NBT®) System in Treatment of Major Depressive Disorder, Register-Study, A. Winkler, E. Tadayon-Manssuri, NEXSTIM Plc.*
- *AVANT Program: Assessing caregivers awareness and knowledge about neurorehabilitation of stroke patients, 2023*
- *Pohl J, Held JPO, Verheyden G, Alt Murphy M, Engelter s, Flöel A, Keller T, Kwakkel G, Nef T, Ward N, Luft AR and Veerbeek JM (2020) Consensus-Based Core Set of Outcome Measures for Clinical Motor Rehabilitation After Stroke – A Delphi Study. Front. Neurol. 2020 11:875*
- *Rivaroxaban Versus Aspirin in Secondary Prevention of Stroke and Prevention of Systemic Embolism in Patients With Recent Embolic Stroke of Undetermined Source (ESUS) „NAVIGATE ESUS", NEJM 5. 2018*
- *The Changing Landscape for Stroke Prevention in AF, Findings From the GLORIA-AF Registry Phase 2 Journal of the American College of Cardiology; Volume 69, Issue 7, February 2017*
- *Defining "Advanced" Parkinson's Disease in Clinical Practice: Results from the Austrian Full Analysis set of the OBSERVE-PD Study, a Cross-Sectional Observation Study of 2615 Patients. ÖPG, 2017*

Dr. Strunk – PD & EV-related recent publications:

- ***Discovery of EV corona mediating EV mode of action***
  A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immuno-modulation. Wolf M, Poupardin RW, Ebner-Peking P … Schallmoser K, Volk HD, Strunk D; J Extracell Vesicles 2022; 11(4):e12207; pre-published 20.9.2021, www.biorxiv.org/content/10.1101/808808v2; doi.org/10.1101/808808
- ***Confirmation of EV corona functionality on platelet-derived EVs during organogenesis***
  Synergy of human platelet-derived extracellular vesicles with secretome proteins promotes regenerative functions. Gomes FG, Andrade AC, Wolf M … Meisner-Kober N, Schallmoser K, Strunk D; Biomedicines 2022; 10(2):238
- ***Detection of leukemia-derived EVs***
  Scalable enrichment of immuno-modulatory human acute myeloid leukemia cell line-derived extracellular vesicles. Binder HM, Maeding N, Wolf M, … Huber CG, Schallmoser K, Strunk D; Cells 2021; 10(12):3321
- ***Discovery of EV function during skin organ(oid) regeneration in vitro & in vivo***
  Self-assembly of differentiated progenitor cells facilitates spheroid human skin organoid formation and planar skin re-generation. Ebner-Peking P, Krisch L, Wolf M … Schneeberger A, Schallmoser K, Strunk D; Theranostics 2021; 11(17)8430
- ***Functionality of pluripotent stem cell-derived EVs***
  Hypoxic conditions promote the angiogenic potential of human induced pluripotent stem cell-derived extracellular vesicles. Andrade AC, Wolf M, Binder HM … Zweigerdt R, Schallmoser K, Strunk D; Int J Mol Sci 2021; 22(8):3890
- ***Experimental therapy of spinal cord injury with EVs***
  Extracellular Vesicles Can Deliver Anti-inflammatory and Anti-scarring Activities of Mesenchymal Stromal Cells After Spinal Cord Injury. Romanelli P, Bieler L, Scharler C … Gimona M, Strunk D, Couillard-Despres S; Front Neurol 2019; 10:1225

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